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TITLE: Genetic alterations related to familial alzheimer's disease

Brief Summary Text (21):
The present invention also provides expression vectors comprising a promoter operably linked to one of the nucleic acid molecules described above. Within related aspects, viral vectors are provided that are capable of directing the expression of a nucleic acid molecule as described above. Also provided are host cells which carry the above-described vectors.

Brief Summary Text (22):
The present invention further provides isolated proteins comprising a PS1 gene product, as well as PS1 peptides of greater than 12, 13, or 20 amino acids. Within one embodiment, a protein is provided that has the amino acid sequence set forth in FIGS. 2A-2F. Within another embodiment, the protein is a mutant PS1 gene product that increases the probability of Alzheimer's disease. Such mutants include those with an amino acid substitution at residue 263 (e.g., an arginine:cysteine substitution), or at residue 264 (e.g., a leucine:proline substitution), or at residue 269 (e.g., a histidine:arginine substitution). In addition, PS1 peptides are provided which are composed of 13 to 20 amino acids derived or selected from the N-terminal, internal, or carboxyl-terminal hydrophilic regions.

Brief Summary Text (23):
Within yet another embodiment of the present invention, methods of treating or preventing Alzheimer's disease are provided, comprising the step of administering to a patient a vector containing or expressing a nucleic acid molecule, protein, or antibody specific for a PS1 protein as described above, thereby reducing the likelihood or delaying the onset of Alzheimer's disease in the patient. Within certain embodiments, the above methods may be accomplished by in vivo administration.

Brief Summary Text (26):
The present invention further provides nucleic acid probes which are capable of specifically hybridizing (as defined below) to a PS1 gene under conditions of high stringency. Within one related aspect, such probes comprise at least a portion of the nucleotide sequence shown in FIGS. 1A-1F or 2A-2F, or its complementary sequence, the probe being capable of specifically hybridizing to a mutant PS1 gene under conditions of high stringency. Within one particularly preferred aspect, probes are provided that are capable of specifically hybridizing to a mutant PS1 gene encoding a protein in which amino acid residue 263 is changed from cysteine to arginine, or in which amino acid 264 is changed from proline to leucine, or in which amino acid 269 is changed from arginine to histidine, each under conditions of very high stringency. Representative probes of the present invention are generally at least 12 nucleotide bases in length, although they may be longer. Also provided are primer pairs capable of specifically amplifying all, or a portion of, any of the nucleic acid molecules disclosed herein.

Brief Summary Text (28):
Within another embodiment, methods are provided comprising the steps of: a) obtaining from a patient a biological sample containing nucleic acid, b) amplifying selected nucleic acid sequence associated with a mutant PS1 gene, and c) detecting the presence of an amplified nucleic acid sequence, and thereby determining that the patient has an increased likelihood of contracting Alzheimer's disease.

Brief Summary Text (29):
Within yet another embodiment, methods are provided comprising the steps of: a) contacting a biological sample obtained from a patient with an antibody that

specifically binds to a mutant PS1 protein under conditions and for a time sufficient to allow binding of the antibody to the protein and b) detecting the presence of the bound antibody.

Brief Summary Text (30):
The invention also extends to products useful for carrying out a method of detection, such as DNA probes (labeled or unlabeled), kits and the like. And, the invention also provides a method of detecting a DNA segment within the Alzheimer's disease region of chromosome 14.

Brief Summary Text (33):
Within another embodiment of the present invention, peptide vaccines are provided which comprises a portion of a mutant PS1 gene product containing a mutation, in combination with a pharmaceutically acceptable carrier or diluent.

Brief Summary Text (34):
Within yet another aspect of the invention, transgenic animals are provided whose germ cells and somatic cells contain a PS1 gene which is operably linked to a promoter effective for the expression of the gene, the gene being introduced into the animal, or an ancestor of the animal, at an embryonic stage.

Drawing Description Text (2):
FIGS. 1A-1F depicts the nucleotide sequence of the normal S182 gene, PS1 locus (SEQ ID NO:1). Within the coding region, beneath each line of nucleotide sequence are the corresponding putative amino acid residues (SEQ ID NOs:2 and 7-26).

Drawing Description Text (3):
FIGS. 2A-2F depicts identified mutations (shown by arrows) at nucleotide sequence positions 1035, 1039 and 1054 of the S182 gene, PS1 locus (SEQ ID NO:3). Within the coding region, beneath each line of nucleotide sequence are the corresponding putative amino acid residues (SEQ ID NOs:4 and 7-26).

Detailed Description Text (5):
A "gene" is a DNA sequence related to a single polypeptide chain or protein, and as used herein includes the 5' and 3' ends. The polypeptide can be encoded by a fill-length sequence or any portion of the coding sequence, so long as the functional activity of the protein is retained.

Detailed Description Text (39):
Although one embodiment of the mutant PS1 gene is disclosed in FIGS. 2A-2F, it should be understood that the present invention is not so limited. In particular, within the context of the present invention reference to the PS1 gene should be understood to include derivatives, analogs, or allelic variants of the gene disclosed in FIGS. 1A-1F that are substantially similar. As used herein, a nucleic acid molecule is deemed to be "substantially similar" if (a) the nucleotide sequence is derived from the coding region of the described gene and includes portions of the sequence or allelic variations of the sequences discussed above; (b) the nucleotide sequence is capable of hybridization to nucleotide sequences of the present invention under high or very high stringency (see Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)); or (c) the DNA sequences are degenerate as a result of the genetic code to the DNA sequences defined in (a) or (b).

Detailed Description Text (40):
Further, the PS1 gene includes both complementary and non-complementary sequences, provided the sequences otherwise meet the criteria set forth herein. Within the context of the present invention, high stringency means standard hybridization conditions (e.g., 5.times.SSPE, 0.5% SDS at 65.degree. C., or the equivalent), such that an appropriate nucleotide sequence is able to electively hybridize to nucleotide sequences from the AD-related gene, and to mutant nucleotide sequences. Very high stringency means the nucleotide sequence is able to selectively hybridize to a single allele of the AD-related gene.

Detailed Description Text (42):
Alternately, the PS1 gene may be isolated by PCR methods from genomic DNA, cDNA or

libraries, or by probe hybridization of genomic DNA or cDNA libraries. Primers for PCR and probes for hybridization screening may be designed based on the DNA sequence of PS1 presented herein. The DNA sequence of PS1 and the corresponding predicted amino acid sequence of PS1 is presented in FIGS. 1A-1F. Primers for PCR should be derived from sequences in the 5' and 3' untranslated region in order to isolate a full-length cDNA. The primers should not have self-complementary sequences nor have complementary sequences at their 3' end (to prevent primer-dimer formation). Preferably, the GC content of the primers is about 50% and contain restriction sites. The primers are annealed to cDNA and sufficient cycles of PCR are performed to yield a product readily visualized by gel electrophoresis and staining. Mutations can be visualized by single strand conformation polymorphism (SSCP) analysis. The amplified fragment is purified and inserted into a vector, such as .lambda.gt10 or pBS(M13+), and propagated.

Detailed Description Text (62):
Antigenic epitope-bearing peptides and polypeptides of the invention designed according to the above guidelines preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of a polypeptide of the invention, containing about 30 to about 50 amino acids, or any length up to and including the entire amino acid sequence of a polypeptide of the invention, also are considered epitope-bearing peptides or polypeptides of the invention and also are useful for inducing antibodies that react with the mimicked protein. Preferably, the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (i.e., the sequence includes relatively hydrophilic residues and highly hydrophobic sequences are preferably avoided); and sequences containing proline residues are particularly preferred.

Detailed Description Text (64):
Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. For instance, Geysen et al., supra, discloses a procedure for rapid concurrent synthesis on solid supports of hundreds of peptides of sufficient purity to react in an enzyme-linked immunosorbent assay. Interaction of synthesized peptides with antibodies is then easily detected without removing them from the support. In this manner a peptide bearing an immunogenic epitope of a desired protein may be identified routinely by one of ordinary skill in the art. For instance, the immunologically important epitope in the coat protein of foot-and-mouth disease virus was located by Geysen et al. with a resolution of seven amino acids by synthesis of an overlapping set of all 208 possible hexapeptides covering the entire 213 amino acid sequence of the protein. Then, a complete replacement set of peptides in which all 20 amino acids were substituted in turn at every position within the epitope were synthesized, and the particular amino acids conferring specificity for the reaction with antibody were determined. Thus, peptide analogs of the epitope-bearing peptides of the invention can be made routinely by this method. U.S. Pat. No. 4,708,781 to Geysen (1987) further describes this method of identifying apeptide bearing an immunogenic epitope of a desired protein.

Detailed Description Text (81):
Techniques for transforming fungi are well known in the literature, and have been described, for instance, by Beggs (Nature 275:104-108 (1978)), Hinnen et al. (Proc. Natl. Acad. Sci. USA 75:1929-1933 (1978)), Yelton et al. (Proc. Natl. Acad. Sci. USA 81:1740-1747 (1984)), and Russell (Nature 301:167-169, 1983)). The genotype of the host cell may contain a genetic defect that is complemented by the selectable marker present on the expression vector. Choice of a particular host and selectable marker is well within the level of ordinary skill in the art.

Detailed Description Text (82):
Viral vectors include those which comprise a promoter that directs the expression of an isolated nucleic acid molecule that encodes an Alzheimer disease protein. A wide variety of promoters may be utilized within the context of the present invention, including for example, promoters such as MoMLV LTR, RSV LTR, Friend MuLV LTR, adenoviral promoter (Ohno et al., Science 265:781-784 (1994)), neomycin phosphotransferase promoter/enhancer, late parvovirus promoter (Koering et al., Hum.

Gene Therap. 5:463 (1994)), herpes tk promoter, SV40 promoter, metallothionein enhancer/promoter, cytomegalovirus immediate early promoter, and the cytomegalovirus immediate late promoter.

Detailed Description Text (83):
Within particularly preferred embodiments of the invention, the promoter is a tissue-specific promoter (see e.g., WO 91/02805; EP 0,415,731; and WO 90/07936). Representative examples of suitable tissue specific promoters include neural specific enolase promoter, platelet derived growth factor .beta. promoter, bone morphogenetic protein promoter, human .alpha.-1-chimaerin promoter, synapsin I promoter and synapsin II promoter.

Detailed Description Text (84):
In addition, other viral-specific promoters (e.g., retroviral promoters (including those noted above, and others, such as HIV promoters), hepatitis, herpes (e.g., EBV), and bacterial, fungal or parasitic (e.g., malarial)-specific promoters may be utilized in order to target a specific cell or tissue which is infected with a virus, bacteria, fungus or parasite. Thus, PS1 proteins of the present invention may be expressed from a variety of viral vectors. Within various embodiments, either the viral vector itself, or a viral particle which contains the viral vector may be utilized in the methods and compositions described below.

Detailed Description Text (91):
Preferred prokaryotic host cells for use within the present invention include E. coli, Salmonella, Bacillus, Shigella, Pseudomonas, Streptomyces, Streptomyces, and Staphylococcus, as well as many other bacterial genera or species well known to one of ordinary skill in the art. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art (see, e.g., Maniatis et al., supra). Vectors used for expressing cloned DNA sequences in bacterial hosts will generally contain a selectable marker, such as a gene for antibiotic resistance, and a promoter that functions in the host cell. Appropriate promoters include the trp (Nichols & Yanofsky, Meth. Enzymol. 101:155-164 (1983)), lac (Casadaban et al., J. Bacterio. 143:971-980 (1980)), and phage .lambda. (Queen, J. Mol. Appl. Genet. 2:1-10 (1983)) promoter systems.

Detailed Description Text (105):
Mammalian cells which may be useful as hosts include, among others: PCI2, NIE-115 neuroblastoma, SK-N-BE(2)C neuroblastoma, SHSY5 adrenergic neuroblastoma, NS20Y and NG108-15 murine cholinergic cell lines, or rat F2 dorsal root ganglion line, COS (e.g., deposited with the American Type Culture Collection (ATCC) No. CRL 1650 or 1651), BHK (e.g., ATCC No. CRL 6281; BHK 570 cell line (ATCC) under accession number CRL 10314), CHO (ATCC No. CCL 61), HeLa (e.g., ATCC No. CCL 2), 293 (ATCC No. 1573); Graham et al., J. Gen. Virol. 36:59-72 (1977)) and NS-1 cells. Other mammalian cell lines may be used within the present invention, including Rat Hep I (ATCC No. CRL 1600), Rat Hep II (ATCC No. CRL 1548), TCMK (ATCC No. CCL 139), Human lung (ATCC No. CCL 75.1), human hepatoma (ATCC No. HTB-52), Hep G2 (ATCC No. HB 8065), mouse liver (ATCC No. CCL 29.1), NCTC 1469 (ATCC No. CCL 9.1), SP2/0-Ag14 (ATCC No. 1581), HIT-TI5 (ATCC. No. CRL 1777), and RINm 5AHT.sub.2 B (Orskov & Nielson, FEBS 229(1):175-178 (1988)).

Detailed Description Text (106):
Cultured mammalian cells are generally cultured in commercially available serum-containing or serum-free media. Selection of a medium and growth conditions appropriate for the particular cell line used is well within the level of ordinary skill in the art.

Detailed Description Text (114):
A protein is deemed to be "isolated" within the context of the present invention if no other (undesired) protein is detected pursuant to SDS-PAGE analysis followed by Coomassie blue staining. Within other embodiments, the desired protein can be isolated such that no other (undesired) protein is detected pursuant to SDS-PAGE analysis followed by silver staining.

Detailed Description Text (116):
Antibodies to the PS1 proteins may readily be prepared given the disclosure provided

herein. Within the context of the present invention, antibodies are understood to include monoclonal antibodies, polyclonal antibodies, anti-ideotropic antibodies, antibody fragments (e.g., Fab, and F(ab').sub.2, F.sub.V variable regions, or complementarity determining regions). As discussed above, antibodies are understood to be specific against an Alzheimer disease protein if it binds with a K.sub.a of greater than or equal to 10.sup.-7 M, preferably greater than or equal to 10.sup.8 M. The affinity of a monoclonal antibody or binding partner can be readily determined by one of ordinary skill in the art.

Detailed Description Text (119):
Briefly, within one embodiment a subject animal such as a rat or mouse is injected with a PS1 protein or portion thereof as described above. The protein may be admixed with an adjuvant such as Freund's complete or incomplete adjuvant in order to increase the resultant immune response. Between one and three weeks after the initial immunization the animal may be reimmunized with another booster immunization, and tested for reactivity to the protein utilizing assays described above. Once the animal has plateaued in its reactivity to the mutant, it is sacrificed, and organs which contain large numbers of B cells such as the spleen and lymph nodes are harvested.

Detailed Description Text (120):
Cells which are obtained from the immunized animal may be immortalized by transfection with a virus such as the Epstein-Barr virus (EBV) (see, Glasky & Reading, Hybridoma 8(4):377-389 (1989)). Alternatively, within a preferred embodiment, the harvested spleen and/or lymph node cell suspensions are fused with a suitable myeloma cell in order to create a "hybridoma" which secretes monoclonal antibody. Suitable myeloma lines include, for example, NS-1 (ATCC. No. TLB 18), an P3X63-Ag 8.653 (ATCC No. CRL 1580).

Detailed Description Text (125):
Antibodies of the present invention have many uses. For example, antibodies may be utilized in flow cytometry to sort cells bearing such a PS1 protein. Briefly, in order to detect the protein or peptide of interest on cells, the cells are incubated with a labeled monoclonal antibody which specifically binds to the protein of interest, followed by detection of the presence of bound antibody. These steps may also be accomplished with additional steps such as washings to remove unbound antibody. Labels suitable for use within the present invention are well known in the art including, among others, fluorescein isothiocyanate (FITC), phycoerythrin (PE), horse radish peroxidase (HRP), and colloidal gold. Particularly preferred for use in flow cytometry is FITC, which may be conjugated to purified antibody according to known methods.

Detailed Description Text (128):
Assays useful within the context of the present invention include those assays for detecting agonists or antagonists of PS1 protein activity. Other assays are useful for the screening of peptide or organic molecule libraries. Still other assays are useful for the identification and/or isolation of nucleic acid molecules and/or peptides within the present invention, or for diagnosis of a patient with an increased likelihood of contracting Alzheimer's disease.

Detailed Description Text (131):
Mutants of PS1 may be detected by DNA sequence analysis or hybridization with allele-specific oligonucleotide probes under conditions and for time sufficient to allow hybridization to the specific allele. Typically, the hybridization buffer and wash will contain tetramethyl ammonium chloride or the like (see Sambrook et al., Molecular Cloning: A Laboratory Manual, supra).

Detailed Description Text (132):
Probes of the present invention may be composed of DNA, RNA, nucleic acid analogues (e.g., peptide/nucleic acids), or any combination thereof. They may be as small as about 12 nucleotides in length, usually about 14 to 18 nucleotides in length, but may possibly be as large as the entire sequence of a PS1 gene. Selection of probe size is somewhat dependent upon the use of the probe, and is within the skill of the art.

Detailed Description Text (143):
The probes of the present invention can be utilized to detect the presence of PS1 mRNA or DNA within a sample. However, if the nucleic acid is present in only a limited

amount, then it may be beneficial to amplify the relevant sequence such that it may be more readily detected or obtained.

Detailed Description Text (145):
A variety of methods may be utilized in order to amplify a selected sequence, including, for example, RNA amplification (see Lizardi et al., Bio/Technology 6:1197-1202 (1988); Kramer et al., Nature 339:401-402 (1989); Lomeli et al., Clinical Chem. 35(9):1826-1831 (1989); U.S. Pat. No. 4,786,600), and DNA amplification utilizing LCR or polymerase chain reaction ("PCR") (see, U.S. Pat. Nos. 4,683,195, 4,683,202, and 4,800,159) (see also U.S. Pat. Nos. 4,876,187 and 5,011,769, which describe an alternative detection/amplification system comprising the use of scissile linkages), or other nucleic acid amplification procedures that are well within the level of ordinary skill in the art.

Detailed Description Text (149):
Primers for the amplification of a selected sequence should be selected from sequences that are highly specific and form stable duplexes with the target sequence. The primers should also be non-complementary, especially at the 3' end, should not form dimers with themselves or other primers, and should not form secondary structures or duplexes with other regions of DNA. In general, primers of about 18 to 20 nucleotides are preferred, and can be easily synthesized using techniques well known in the art. PCR products, and other nucleic acid amplification products, may be quantified using techniques known in the art, i.e., SSCP analysis.

Detailed Description Text (186):
Cognitive behavior in AD may be measured by any one of several tests (See Gershon et al., Clinical Evaluation of Psychotropic Drugs: Principles and Guidelines, Prien and Robinson (eds.), Raven Press, Ltd., New York, 1994, p. 467). One such test, BCRS, is designed to measure only cognitive functions: concentration, recent memory, past memory, orientation, functioning, and self-care. This test, as well as the Weschler Memory Scale and the Alzheimer's Disease-Associated Scale, may be used to determine improvement following therapeutic treatment. "Improvement" in Alzheimer's disease is present if there is a statistically significant difference in the direction of normality in the Weschier Memory Scale test. For example, test results of the performance of treated patients as are compared to members of the placebo group or between subsequent tests given to the same patient. Improvement within the present invention also encompasses a delay in the age of onset of Alzheimer's disease.

Detailed Description Text (189):
In addition, the pharmaceutical compositions of the present invention may be prepared for administration by a variety of different routes, although intracranial routes are typically preferred. In addition, pharmaceutical compositions of the present invention may be placed within containers, along with packaging material which provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions will include a tangible expression describing the reagent concentration, as well as within certain embodiments, relative amounts of excipient ingredients or diluents (e.g., water, saline or PBS) which may be necessary to reconstitute the pharmaceutical composition.

Detailed Description Text (202):
Within other embodiments of the invention, the vectors which contain or express the nucleic acid molecules which encode a PS1 protein, or even the nucleic acid molecule per se may be administered by a variety of alternative techniques, including for example administration of asialoosomucoid (ASOR) conjugated with poly (L-lysine) DNA complexes (Cristano et al., Proc. Natl. Acad. Sci. USA 92122-92126 (1993)), DNA linked to killed adenovirus (Curiel et al., Hum. Gene Ther. 3(2):147-154 (1992)), cytofectin-mediated introduction (DMRIE-DOPE, Vical, Calif.), direct DNA injection (Acsadi et al., Nature 352:815-818 (1991)); DNA ligand (Wu et al., J. Biol. Chem. 264:16985-16987, 1989); lipofection (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1989)); liposomes (Pickering et al., Circ. 89(1):13-21 (1994); and Wang et al., Proc. Natl. Acad. Sci. USA 84:7851-7855 (1987)); microprojectile bombardment (Williams et al., Proc. Natl. Acad. Sci. USA 88:2726-2730 (1991)); and direct delivery of nucleic acids which encode the PS1 protein alone (Vile and Hart, Cancer Res. 53:3860-3864 (1993)), or utilizing PEG-nucleic acid complexes.

Detailed Description Text (209):
Although early-onset AD is less common than late-onset AD, the PS1 locus is associated with the most aggressive form of the disease (onset 30-60 years), suggesting the importance of mutations in the PS1 locus with regard to causative effects of AD. The PS1 locus has been isolated to the region between D14S53 and D15S58 on human chromosome 14. Within that region, Sherrington et al., Nature 375:754-760 (1995) reported the cloning of a novel gene, S182, with five missense mutations in seven pedigrees segregating early-onset autosomal dominant AD.

Detailed Description Text (216):
Using the SSCA analysis, the sequence obtained from a patient carrying a mutation within the S182 exon can be potentially distinguished from that of a normal control individual. One or more mutation(s) in the S182 exon effecting a conformational change in the secondary/tertiary structure can be quickly visualized in the single stranded molecule. The MDE.TM. gel is designed to permit more compact molecules to run more quickly through the pores of the size differentiating gel, so that a mutated species is revealed as a band in the gel at a different point than that which is consistently seen in normal (control) samples encoded by the same S182 exon region.

Other Reference Publication (38):
Cataldo, A.M. et al., "Lysosomal proteinase antigens are prominently located within senile plaques of Alzheimer's disease: evidence for a neuronal origin," Brain Res. 513(2):181-192 (1990).